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Review article

Gonads and gametogenesis in astigmatic mites (Acariformes: Astigmata)

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ABSTRACT

Astigmatans are a large group of mites living in nearly every environment and exhibiting very diverse reproductive strategies. In spite of an uniform anatomical organization of their reproductive systems, gametogenesis in each sex is highly variable, leading to gamete formation showing many peculiar features and emphasizing the distinct position of Astigmata. This review summarizes the contemporary knowledge on the structure of ovaries and testes in astigmatic mites, the peculiarities of oogenesis and spermatogenesis, as well as provides new data on several species not studied previously. New questions are discussed and approaches for future studies are proposed.

1. Introduction

Mites (incl. ticks) consist of a large group of chelicerate arthropods comprising more than 55 000 described species, however, acarologists estimate that many times this number have yet to be described (Walter and Proctor, 1999; Krantz, 2009). In the last decades mites (Acari) were considered to be a natural taxon; however, diphyly has also been postulated (Zakhvatkin, 1952; Van der Hammen, 1977, 1979, 1989). Quite recently, a molecular study on the phylogeny of Acari again strongly supported its diphyletic origin (Dabert et al., 2010). Consequently, there are two independent branches (superorders): Parasitiformes (=Anactinotrichida) and Acariformes (=Actinotrichida). The latter originated at least in the Early Devonian (Hirst, 1923; Norton et al., 1988; Bernini, 1991) and comprises four taxa: the Trombidiformes (order), the Endeostigmata (suborder), the Oribatida (suborder), and the cohort Astigmata (= Astigmatina, Acaridida) (Lindquist et al., 2009). Astigmata is considered a natural group and most likely derived from an early clade (infraorder, acc. to Schatz et al., 2011) of Oribatida, the Desmonomata (OConnor, 1984; Norton, 1998).

1.1. Systematics of Astigmata

As currently recognized (OConnor, 2009), the astigmatic lineage diversified into 10 superfamilies, 71 families comprising 960 genera and more than 6100 described species (Klimov and OConnor, 2013). At present, even at a high taxonomic level the suggested phylogenetic relationships are based on traditional morphological analyses rather than molecular studies, with the exception of a recent molecular study by Klimov and OConnor (2013). A provisional but still temporarily accepted cladogram is presented in Fig. 1 by OConnor (2009), adopted from earlier work (Norton et al., 1993). It shows the phylogenetic relationships of Astigmata superfamilies and combines Pterolichoidea, Analgoidea and Sarcoptoidea into a monophyletic group Psoroptidia with ca. 3800 species (OConnor, 1982; Klimov and OConnor, 2008), although the composition of Pterolichoidea and Analgoidea is somewhat disputable (for details see: Proctor, 2003).

1.2. Environment of Astigmata

Although astigmatan free-living mites are abundant in wet litter and soil, especially in highly decomposed material, they often are the dominant mite group in patchy or ephemeral habitats. They are numerous in decaying organic matter, dung, carrion, sap flows, dry

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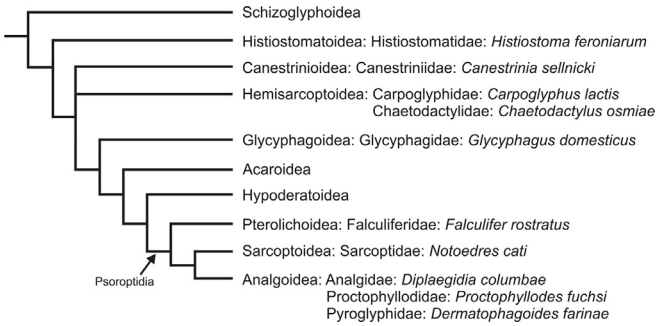

Fig. 1. Cladogram of Astigmata superfamilies (adopted from Norton et al. (1993) by OConnor (2009) showing families and species studied originally for this review.

and water-filled tree holes, phytotelmata and caves (Hughes, 1976; Evans, 1992; Fashing, 1994, 1998). The other species are successful in destroying stored food such as cereals, flour, cheese, dried fruits and meat, etc., leading to considerable crop damage (Hughes, 1976). An abundance of Astigmata is associated with other animals, mainly insects and amniotic vertebrates (e.g. all Psoroptidia with the exception of Pyroglyphidae which are free-living), frequently as serious dermicolous parasites (Fain and Lukoschus, 1986; Proctor, 2003; OConnor, 2009). They can also inhabit bird and mammalian nests or such distinct habitat as feathers. Despite a wide range of habitats and a successful adaptive radiation, these mites share many distinct features in reproductive anatomy and reproductive behavior.

2. Materials and methods

In this review, original results were obtained through routine transmission electron microscopy (TEM) with a procedure summarized as follows. The sex of studied mite species (Histiostomatidae: *Histiostoma feroniarum* Dufour, Canestriniidae: *Canestrinia sellnicki* (Samšiňák), Carpoglyphidae: *Carpoglyphus lactis* L., Chaetodactylidae: *Chaetodactylus osmiae* (Dufour), Glycyphagidae: *Glycyphagus domesticus* (De Geer), Falculiferidae: *Falculifer rostratus* (Buhcholz), Sarcoptidae: *Notoedres cati* Hering, Analgidae: *Diplaegidia columbae* (Buhcholz), Proctophyllodidae: *Proctophyllodes fuchsi* Mironov, Pyroglyphidae: *Dermatophagoides farinae* Hughes) (Fig. 1) was identified under a Nikon SMZ1000 stereomicroscope (Nikon Instruments Europe, Amsterdam, Netherlands). After immersion into a droplet of Karnovsky's fixative (Karnovsky, 1965) (mixture containing 2% formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2) on a Parafilm-coated microscopic slide, the anterior part of the body was cut off with a fine razor blade and the remaining rear part was transferred into fresh fixative for 24 h at 4° C. Fixed material was rinsed 4 × 15 min in 0.1 M cacodylate buffer containing 8% sucrose and postfixed with 1.4% osmium tetroxide in 8% sucrose overnight at 4° C. The specimens were then washed and dehydrated in a graded ethanol series followed by propylene oxide and embedded in Epon™ 812 substitute (Sigma–Aldrich).

Semithin cross sections were stained with an Azur II and methylene blue (1:1) mixture, whereas thin sections were collected on formvar coated grids, contrasted with uranyl acetate and lead citrate according to standard protocols (Venable and Coggeshall, 1965), and examined under a transmission electron microscope JEOL JEM 100SX (JEOL Ltd., Tokyo, Japan) at 80 kV in the Department of Cell Biology and Imaging, Institute of Zoology, Jagiellonian University.

For fluorescent staining with DAPI and Pyronin Y, the females of *Rhizoglyphus echinopus* were processed as follows: the posterior parts of mite bodies were fixed for 2 h at 4° C in fixative containing 4% paraformaldehyde and 2.5% sucrose in 0.01 M PBS. After fixation, the material was washed and dehydrated in a graded ethanol series then embedded in LR-White (Fluka) resin. Semithin sections were stained 30 min with DAPI, washed several seconds in PBS, and stained 20 min with Pyronin Y (Sigma–Aldrich)(20 μg/ml). After brief washing in PBS sections were mounted and examined under a fluorescence microscope Olympus BX51 (Olympus Corporation, Tokyo, Japan) fitted with appropriate filters.

3. Reproduction of Astigmata

Both sexual and parthenogenetic species occur in Astigmata, the latter represented by arrhenotokous, thelytokous, and rare deuterotokous organisms. In species possessing both females and males (sexual and arrhenotokous species) the proportion of sexes may be only slightly biased towards females. The males use their intromittent organ, the aedeagus (=penis) to inseminate females with sperm during copulation. Insemination never occurs through the oviporus, but via a supplementary inseminatory system. Sexual species (with diploid females and diploid males) occur for instance in Acaroidea and Glycyphagoidea, whereas arrhenotokous diplohaploid species (with diploid females and haploid males) are known in Histiostomatoidea (Histiostomatidae: *Histiostoma*), Hemisarcoptoidea (Winterschmidtiidae: *Kennethiella, Ensliniella, Kurosaia*) (Hughes and Jackson, 1958; Heinemann and Hughes, 1969; Cowan, 1984; Klompen et al., 1987; Okabe and Makino, 2003) and Sarcoptoidea. Thelytokous species occur in Histiostomatoidea (Histiostomatidae), Acaroidea (e.g. Acaridae: *Schwiebea*) (Okabe and OConnor, 2001; Okabe et al., 2008). Thelytokous populations are composed of females but extremely rare males can also be occasionally found; such males are non-reproducing as is believed, but their reproductive systems and/or spermatozoa have never been studied. In *Knemidocoptes mutans* (Analgoidea: Epidermoptidae) the frequency of males in populations is 2–4% (Dubinin, 1953); such strong bias towards males in practically sedentary mites suggests thelytoky rather than the effect of local mate competition, a phenomenon which can also lead to sex ratio distortion (Hamilton, 1967). Deuterotoky, in which both males and females are produced from unfertilized eggs, has been reported in Histiostomatidae (Heinemann and Hughes, 1969).

In most cases, the type of reproduction is only suspected and is based mainly on population structure since detailed studies are usually missing. Moreover, some phenomena concerning reproduction can be misinterpreted. For instance, a well-known cosmopolitan species, *H. feroniarum* (Histiostomatidae), has been for years believed to be comprised of arrhenotokous and thelytokous populations (Hughes and Jackson, 1958). Recent unpublished molecular studies (in co-operation with Dr. Mirosława Dabert, Adam Mickiewicz University, Poznań, Poland) revealed that there are two separate but morphology practically indistinct species, an arrhenotokous species (*H. feroniarum*) and a thelytokous form (*Histiostoma* sp.).

4. Reproductive systems

The anatomy of reproductive systems in Astigmata has been studied using light-microscopy since the 19th century (Nalepa, 1884, 1885; Michael, 1901; Hughes and Hughes, 1938; Dubinin, 1953; Hughes, 1959; Popp, 1967; Rohde and Oemick, 1967; Prasse, 1968, 1970; Heinemann and Hughes, 1970; Kuo and Nesbitt, 1970; Woodring and Carter, 1974; Vijayambika and John, 1975; Griffiths and Boczek, 1977; Baker and Krantz, 1985; Witaliński and Walzl, 1995), and has also been studied at the ultrastructural level (Witaliński et al., 1990; Walzl, 1992; Desch, 2001; Walzl et al., 2004;

Lekimme et al., 2005; Klien and Walzl, 2010; Witaliński et al., 2014). Some external details of female and male reproductive organs were also described using scanning electron microscopy (SEM) in pyroglyphid mites (Mariana et al., 2008).

Finally, it should be emphasized that the most basal astigmatan group, Schizoglyphoidea, has not been studied since only several immature specimens of one species are known (OConnor, 2009), whereas living adult males and females are required for a comprehensive description of reproductive anatomy. Therefore, in this mini-review on anatomical and cytological aspects of reproduction, the superfamily Histiostomatoidea is treated as the most basal in Astigmata.

4.1. The female reproductive system

Reproductive systems in female astigmatans studied so far are rather uniform and composed of two parts, the oogenetic (primary) and the inseminatory (secondary) parts (Griffiths and Boczek, 1977; Witaliński et al., 1990; Witaliński and Walzl, 1995) (Fig. 2A–D). The oogenetic part comprises paired ovaries and oviducts, an unpaired common oviduct (sometimes also called the *uterus*, e.g. Prasse, 1970; Witaliński et al., 1990), a cuticle-lined preoviporal chamber (preoviporal canal of Witaliński et al., 1990), and the oviporus. The oviporus is a complex cuticular structure comprising eugenital lips (internal paragynal folds of Witaliński et al., 1990) covering the eugenital orifice, genital papillae in diachilous slits, anterolateral progenital lips (external paragynal folds of Witaliński et al., 1990), and a medial (epigynal) lip; the progenital chamber (*sensu* Van der Hammen, 1980) is the space between the eugenital lips and anterolateral progenital lips (for details see: Prasse, 1970; Van der Hammen, 1980, 1989; Witaliński et al., 1990; Evans, 1992; Alberti and Coons, 1999).

Spherical or subspherical ovaries are located symmetrically in the rear part of the idiosoma, usually on both sides of the last section of the alimentary tract, i.e. the postcolon, anal atrium and anus. They occupy a considerable part of the idiosoma, but in *C. sellnicki* are very small, spherical and located directly above the ventral cuticle (unpublished). In Psoroptidia, ovaries are located dorsally as in *Sarcoptes scabiei* and *N. cati* – Sarcoptidae (Witaliński and Walzl, 1995), *Psoroptes* spp. – Psoroptidae (Lekimme et al., 2005) (Fig. 2B–D), and *F. rostratus* – Falculiferidae, but in the latter species they are additionally shifted anteriorly (Fig. 4D). Oviducts emerge at the ventral, anteroventral or lateroventral surface of the ovaries and run forward either straight to the oviporal region of the mite body as in the sarcoptid mites: *S. scabiei* and *N. cati* (Witaliński and Walzl, 1995), or are S-shaped bending twice to reach the oviporal region as in *Acarus siro*, *Tyrophagus perniciosus*, or *Sancassania berlesei* (Acaridae) (Walzl et al., 2004)

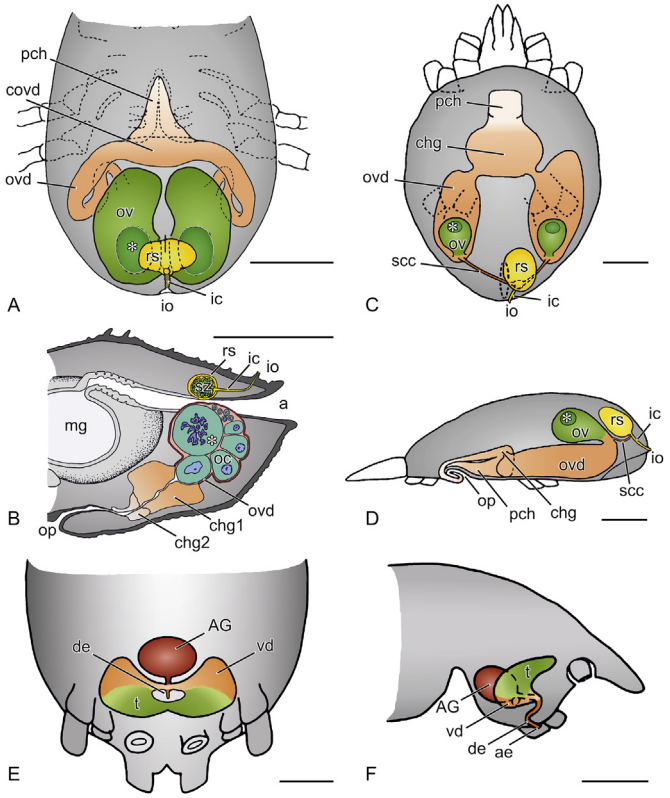

Fig. 2. Schematic representation of reproductive systems in females (A–D) and males (E, F) of Astigmata mites. (A) *Acarus siro* (Acaridae) dorsal view, (B) *Sarcoptes scabiei* (Sarcoptidae) axial section, (C, D) *Psoroptes ovis* (Psoroptidae) female, dorsal (C) and lateral (D) view, (E, F) *Psoroptes ovis* male, ventral (E) and lateral (F) view. Based on Witaliński et al. (1990) (A), Desch (2001) (B), Lekimme et al. (2005) (C–F). Abbreviations: asterisk – location of the ovarian nutritive cell (ONC), a – anus, ae – aedeagus, AG – male accessory gland, chg – chorional gland, chg1, 2 – two parts of chorional gland in *Sarcoptes*, covd – common oviduct, de – ejaculatory duct, ic – inseminatory canal, io – inseminatory opening, mg – midgut, oc – oocyte, op – oviporus, ov – ovary, ovd – oviduct, pch – preoviporal chamber, rs – spermatheca, scc – sperm-conveying cord, sz – spermatozoa in spermatheca, t – testis, vd – deferent duct. Scale bars: 100 μm in (A, B, E, F); 200 μm in (C, D).

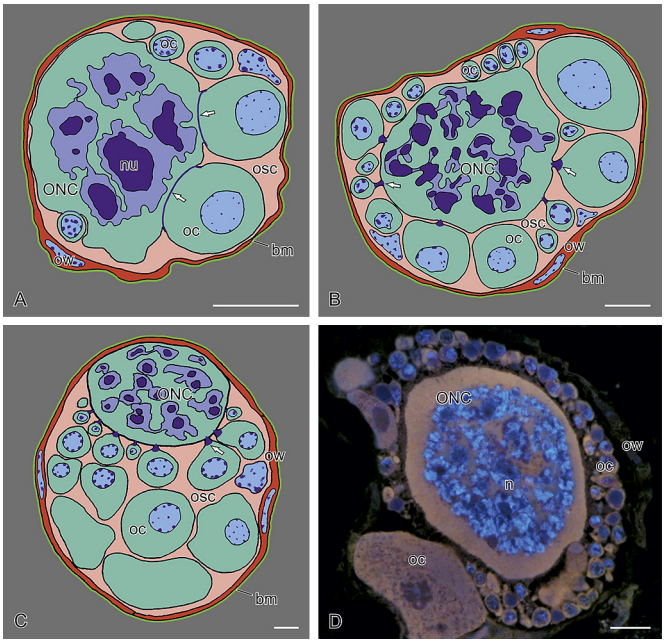

Fig. 3. Ovary organization as visible on cross sections of female mites (A–C) and fluorescent staining of cross-sectioned ovary (D). (A) Ovary in *Histiostoma* spp. showing eccentric ONC with ramified nucleus and large nucleoli (nu). Oocytes in various developmental stages (oc) are connected with the ONC via intercellular bridges with diaphragm-crossed lumina (arrows). Germinal line cells are embedded in somatic ovarian stroma cells (osc). Thin ovarian wall cells (ow) lying on basal lamina (bm) encompass the ovary. (B) In the ovary of Acaridae and Carpoglyphidae the ONC is located subcentrally and oocytes (oc) are connected with the ONC via funnel-type intercellular bridges filled by electron-dense material (arrows). Other abbreviations as in (A). (C) The ONC in the ovary of *Glycyphagus* is in a dorsal position whereas the ventral part of the gonad contains oocytes (oc) in different developmental stages. Other abbreviations as in (A). (D) The LR-White semithin section through the ovary in *Rhizoglyphus echinopus* stained sequentially with DAPI and pyronine Y. DAPI (blue) reveals DNA whereas pyronine Y counterstains RNA. Note very strong signal for DNA from the ONC nucleus as compared to oocyte (oc) nuclei. oc – oocytes, ONC – the ovarian nutritive cell, ow – ovarian wall cells. Scale bars: 10 μm.

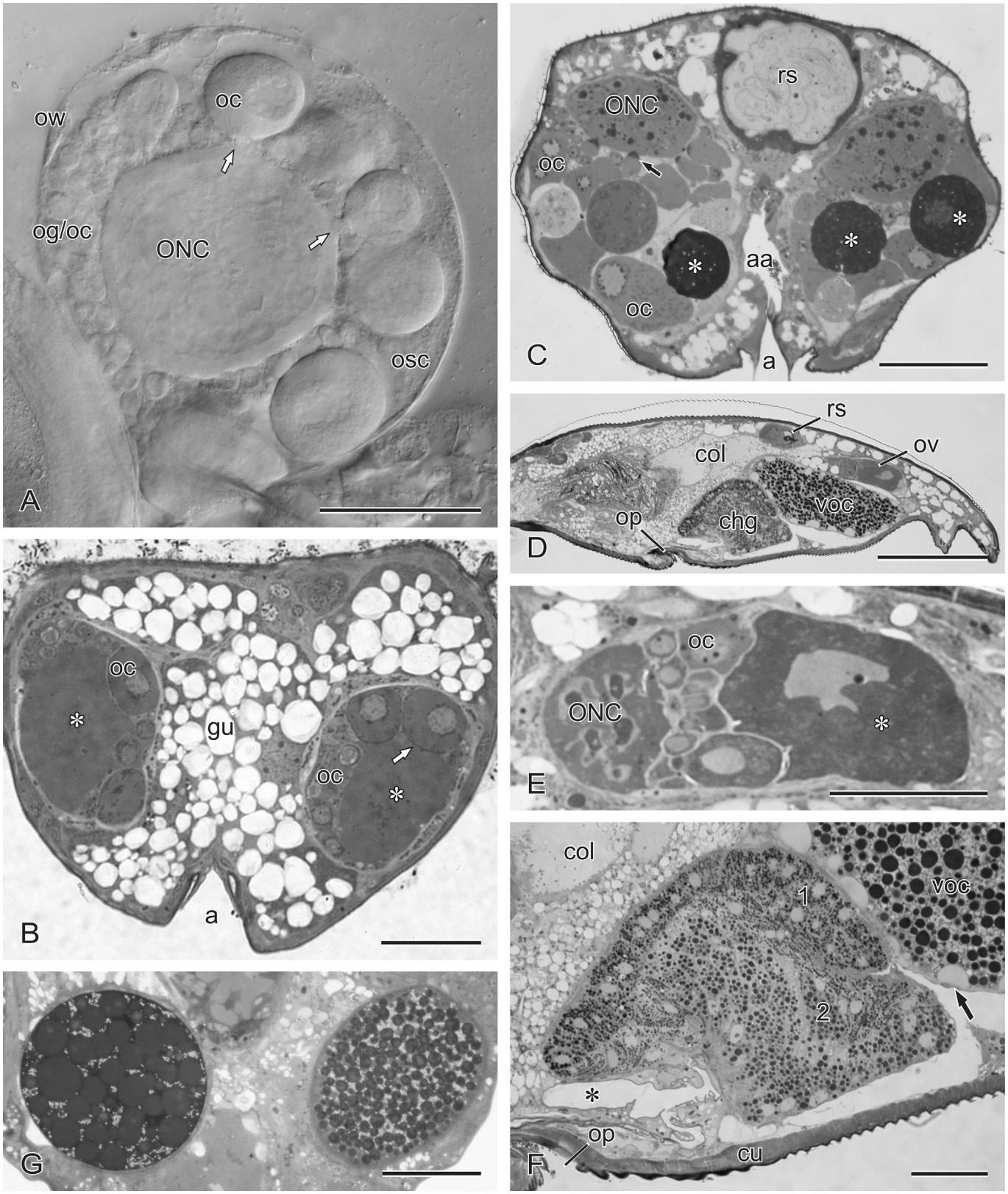

Fig. 4. Differential interference contrast (A) and conventional light microscopy (B–G). (A) Dissected ovary from *Rhizoglyphus echinopus* showing the ONC, oogonia/early oocytes (og/oc) and developmentally advanced oocytes (oc) embedded in ovarian stroma cells (osc). Ovarian wall cells (ow) and intercellular bridges (arrows) are also visible. (B) Semithin cross section through a *Histiostoma feroniarum* female. Ovaries contain ONCs (asterisks) surrounded at other than abaxial sides by the oocytes (oc). Arrow points to diaphragm-type intercellular bridge. (C) Semithin cross section through *Glycyphagus domesticus* female. The ONC is located dorsally whereas the rest of the ovary is filled with oocytes (oc) in different stages of development. Vitellogenic oocytes just before entering the oviducts are also visible (asterisks). Arrow indicates intercellular bridge. (D) Axial semithin section of *Falculifer rostratus* female; anterior end of the body directed to the left. Spermatheca (rs), ovary (ov), vitellogenic oocyte (voc) in the oviduct, as well as chorional gland (chg) close to oviporus (op) are visible. Note lenticular extensions of perivitelline space in the vitellogenic oocyte. (E) The ovary in *F. rostratus* sectioned as in (D). The ONC, early oocytes (oc) and

(Fig. 2A). Ovaries in *F. rostratus* are located both dorsally and more anteriorly, while oviducts emerge to run posteriorly and then turn ventrally toward the oviporal region of the idiosoma. Consequently, in *F. rostratus* two distinct parts of the oviduct are present: a proximal upper section and a distal lower section. In all species, the oviducts close to the oviporus coalesce to form an unpaired common oviduct (=uterus or chorion gland in sarcoptid and psoroptid mites – Fig. 4D and F). The common oviduct passes forward into a preoviporal chamber (sometimes incorrectly termed vagina), lined with a thin, usually plicated cuticle. This part can form a short-tube ovipositor by eversion which allows precise egg manipulation during oviposition. The genital aperture, the oviporus, is a longitudinal, transversal, or inverted V-shaped slit located in the mid region of the mite venter. Its structure is complex because the cuticular walls are folded and two pairs of finger-like genital papillae are located between folds (for details see: Witaliński et al., 1990; Evans, 1992; Walzl, 1992; Alberti and Coons, 1999). The pregenital sclerite (epigynum) rarely occurs in free-living astigmatans (many Glycyphagidae) but is common in parasitic Astigmata (Ehrnsberger et al., 2001).

Female accessory glands occurring as paired, separate structures connected with the oviducts are absent. Paired accessory glands have been reported in some species (Kuo and Nesbitt, 1970; Baker and Krantz, 1985; Witaliński et al., 1990), but subsequent studies with serial semithin sections have shown that the interpretation of the distal third portion of the oviduct as separate accessory glands was erroneous (Witaliński, 1993; Witaliński and Walzl, 1995). However, in non-psoroptidian species the distal third portion of the oviducts has a thickened glandular wall, whereas in psoroptidians the common oviduct forms dorsally a large, more or less distinct glandular pocket, the chorion gland. In *S. scabiei* and *N. cati* the chorion gland comprises only one type of secretory cells, but in *F. rostratus* it contains two types of cells located in two distinct regions (Fig. 4D and F) (Witaliński, 1993).

The inseminatory part (Fig. 2A–D) starts with a copulatory or inseminatory opening (*bursa copulatrix*) located at the rear end of the idiosoma as in Acaridae, Carpoglyphidae and many other stored food pests; the copulatory opening is frequently situated in a shallow depression formed by thickened cuticle (e.g. Acaridae, Suidasiidae, Carpoglyphidae, Falculiferidae and many other Psoroptidia)(Witaliński et al., 1990; Witaliński and Walzl, 1995; Ahamad et al., 2011). In some groups (e.g. Histiostomatoidea: Histiostomatidae: *H. feroniarum, Histiostoma* sp., Hemisarcoptoidea: Chaetodactylidae: *C. osmiae*) the bursa copulatrix forms a tube or, as in Glycyphagoidea: Glycyphagidae, a cuticular collar protruding from the hind end of the body. An especially long caudal protrusion of the bursa copulatrix/inseminatory canal is present in some Pterolichoidea (Crypturoptidae and some Caudiferidae) (Gaud, 1982; Proctor, 2003). Instead terminally, an inseminatory opening can be located more dorsally and placed at the apex of conical, several micrometer long cuticular papilla (e.g. Psoroptoidea: Psoroptidae: *Psoroptes* spp. – Lekimme et al., 2005; Sarcoptoidea: Sarcoptidae: *S. scabiei* – Desch, 2001, and *N. cati*) (Fig. 2B–D). The type of bursa copulatrix is correlated with the shape of the apex of the aedeagus which is either pointed when matched with a concaved inseminatory opening (Prasse, 1970; Witaliński et al., 1990; Ahamad et al., 2011), or is modified and concaved terminally to firmly accept the insertion of the copulatory papilla (Sarcoptidae).

From the copulatory opening, an inseminatory canal (internal diameter ranges from 0.5 to 0.6 μm to several micrometers – Witaliński and Walzl, 1995; Desch, 2001) leads to the basal part of the spermatheca (seminal receptacle, *receptaculum seminis*) where sperm is stored before migration to ovaries via sperm-conveying cords (Fig. 2C and D). The inseminatory canal has a cuticular lining, sometimes with additional external taenidia-like strengthening as in *A. siro* (Witaliński et al., 1990), *Pterodectes* sp. (Popp, 1967), *S. scabiei* (Desch, 2001) and *Psoroptes* spp. (Lekimme et al., 2005), and varies considerably in length. In non-psoroptidians it is short or moderately long (15–20 μm in *H. feroniarum* – orig., 14 μm in *A. siro* – Witaliński et al., 1990; 62–65 μm in *Caloglyphus* (=*Sancassania*) *berlesei* and 43–47 μm in *G. domesticus* – Witaliński and Walzl, 1995), but in Psoroptidia it is usually long (70–75 μm in *N. cati* – Witaliński and Walzl, 1995)(ca. 70 μm in *S. scabiei* – Witaliński and Walzl, 1995, 50 μm in *D. farinae*, 100 μm in *Pterolichus obtusus*, *Grallolichus proctogamus,* 130 μm in *Pseudolichus phasiani* – Liana, 2004). The inseminatory canal in *F. rostratus* is extremely long (290–300 μm) and narrow, with a lumen diameter of 0.6 μm. According to Dubinin's (1953) illustration, the inseminatory canal in *Trouessartia rosterii* is also very long, but it is short in other analgesoid feather mites such as *Bdellorhynchus polymorphus* and *Analges passerinus*.

Sperm-conveying cords are solid cellular structures, conical in *A. siro,* but thin and cord-like in most other species in which the spermatheca is placed far from the ovaries, as, for instance, in *F. rostratus* and *P. fuchsi* where their diameter is 4.0–4.5 μm and ca. 3.0 μm, respectively. Spermatozoa migrate between conveying cord cells (Fig. 6A) from the basal part of the spermatheca towards the ovaries (*A. siro* – Witaliński et al., 1990; *F. rostratus* – unpublished). Syngamy occurs within the ovary with previtellogenic oocytes before they are covered by a vitelline envelope; consequently, in sperm cells of Astigmata the acrosome is absent (Liana and Witaliński, 2005).

The spermatheca in Astigmata (Figs. 2A, D and 4C, D) (Witaliński et al., 1990; Desch, 2001; Lekimme et al., 2005) is a complex saccular organ which consists of cellular and cuticular elements. The cuticular lining of the inseminatory canal amalgamates with the solid cuticular basal part of the spermatheca, the main part of the spermatheca visible in mites on microscopical slides examined under a light microscope. The cuticle of the basal part is supported by a thick layer of cells which continue anteriorly to form the saccular part of the spermatheca. The wall of the saccular part is thin and its cells form many long, internally projecting microvilli. The margin of the basal part of the spermatheca protrudes to form two very thin, more or less continuous, fine cuticular lamellae which encompass the internal space of the spermatheca. Thus, the lumen of the spermatheca is divided into two compartments, (1) an external one, penetrated with microvilli of spermatheca cells, and (2) an internal one, delimited by double lamellae, to which sperm and other male-derived substances are introduced. It is believed that sperm cells leave the spermatheca basis to enter conveying cords and then ovaries via a pair of openings in the basal part of the spermatheca located near two minute V-shaped cuticular appendages visible in light microscopical images. Klien and Walzl (2010) demonstrated that in *S. berlesei,* sperm cells aggregate in the spermatheca close to the entrance into such V-shaped appendages. These appendages,

advanced previtellogenic oocyte (asterisk) are sequentially distributed from the anterior to posterior end of the ovary. (F) Chorional gland in *F. rostratus* sectioned as in (D), showing two types of glandular cells (1 and 2), preoviporal chamber (asterisk) and oviporal opening (op) in the ventral cuticle (cu). Vitellogenic oocyte (voc) contains dark yolk spheres and lenticular extensions of perivitelline space (arrow). (G) Sections of two oviductal oocytes in *Chaetodactylus osmiae*. The right oocyte contains numerous smaller yolk spheres than the left one, much advanced in vitellogenesis. Light profiles of lipid droplets occur between the yolk spheres. a – anal slit, aa – anal atrium, col – colon, gu – guanine, rs – spermatheca. Scale bars: 50 μm in (A, C, G); 100 μm in (D), 20 μm in (B, E, F).

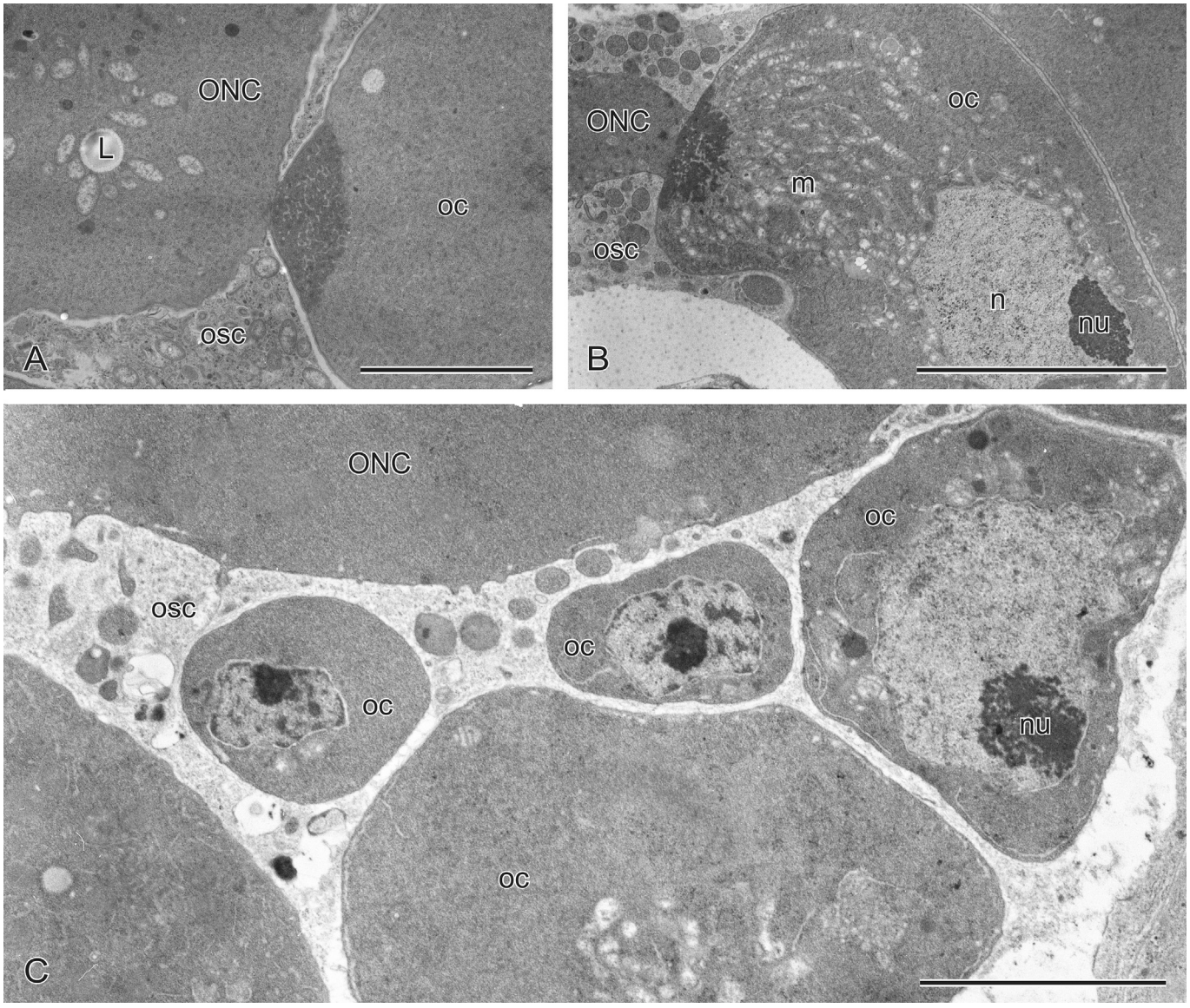

Fig. 5. Details of the ovaries in TEM. (A) *Dermatophagoides farinae*. Funnel-type intercellular bridge filled with an electron-dense granular material connecting the ONC and oocyte (oc). The ONC contains elongated inclusions of microorganismal appearance surrounding the lipid droplet (L); similar inclusions are abundant in the ovarian stroma cell (osc). (B) *Glycyphagus* sp. Funnel-type intercellular bridge (sectioned somewhat out of axis) between a protrusion of the ONC and previtellogenic oocyte (oc). Mitochondria (m) in the oocyte are radially aggregated in the vicinity of the electron-dense bridge material and around the nucleus (n). (C) *Glycyphagus domesticus*. The ONC surrounded by oocytes (oc). Note spherical protrusions of the ONC embedded in the ovarian stroma cell (osc) close to the ONC surface. n – nucleus, nu – nucleolus, osc – ovarian stroma cell. Scale bars: 5 μm in (A, C); 10 μm in (B).

named the ducti conjunctivi, consist of a sclerotized part, the funnel, which is ca. 40 μm long. Its diameter varies from 1 μm at the spermatheca to 20 μm at the distal end. These interesting structures, however, are still waiting for precise descriptions at the ultrastructural level.

4.1.1. The ovary

The ovaries in Astigmata (Fig. 3) are of nutrimental type and, as in other animals, are composed of two elements: germinal and somatic cells. Oogonia and previtellogenic oocytes belong to germinal cells, as well as one very large and spherical cell of nutritive nature, the so-called ovarian central cell. All these germline cells are embedded in only several somatic cells with peripherally positioned nuclei. The ovary is surrounded by a layer of thin epithelium which is difficult to discern under a light microscope and, e.g., is not mentioned in the sarcoptid mite *N. cati* (Witaliński, 1988). Vitellogenic oocytes occur in the transient zone between the ovary and oviduct and fill the oviductal lumen, in which vitellogenesis is completed and egg envelopes begin to form.

Prasse (1968) was the first to observe the ovarian central cell and later observations confirmed its presence in all studied species (Witaliński et al., 1990; Walzl, 1992; Witaliński and Walzl, 1995; Desch, 2001; Lekimme et al., 2005; Schwaha et al., 2008; Witaliński et al., 2014). Indeed, the central cell in many species is located centrally or subcentrally (*A. siro, R. echinopus, C. sellnicki*) within the ovary (Fig. 3B and D), but in some cases is shifted adaxially, as in *H. feroniarum* (Figs. 3A and 4B), *Histiostoma* sp., abaxially as in *C. lactis,* abaxially and anteriorly as in *Psoroptes* spp. (Lekimme et al., 2005), antero-dorsally as in *S. scabiei* (Desch, 2001), dorsally as in *N. cati,* or is located either dorsally or subcentrally as in *G. domesticus* (Figs. 3C and 4C). Since it can actually take central or quite eccentric positions, I propose to use the term ovarian nutritive cell (ONC), as a more proper name instead of central cell.

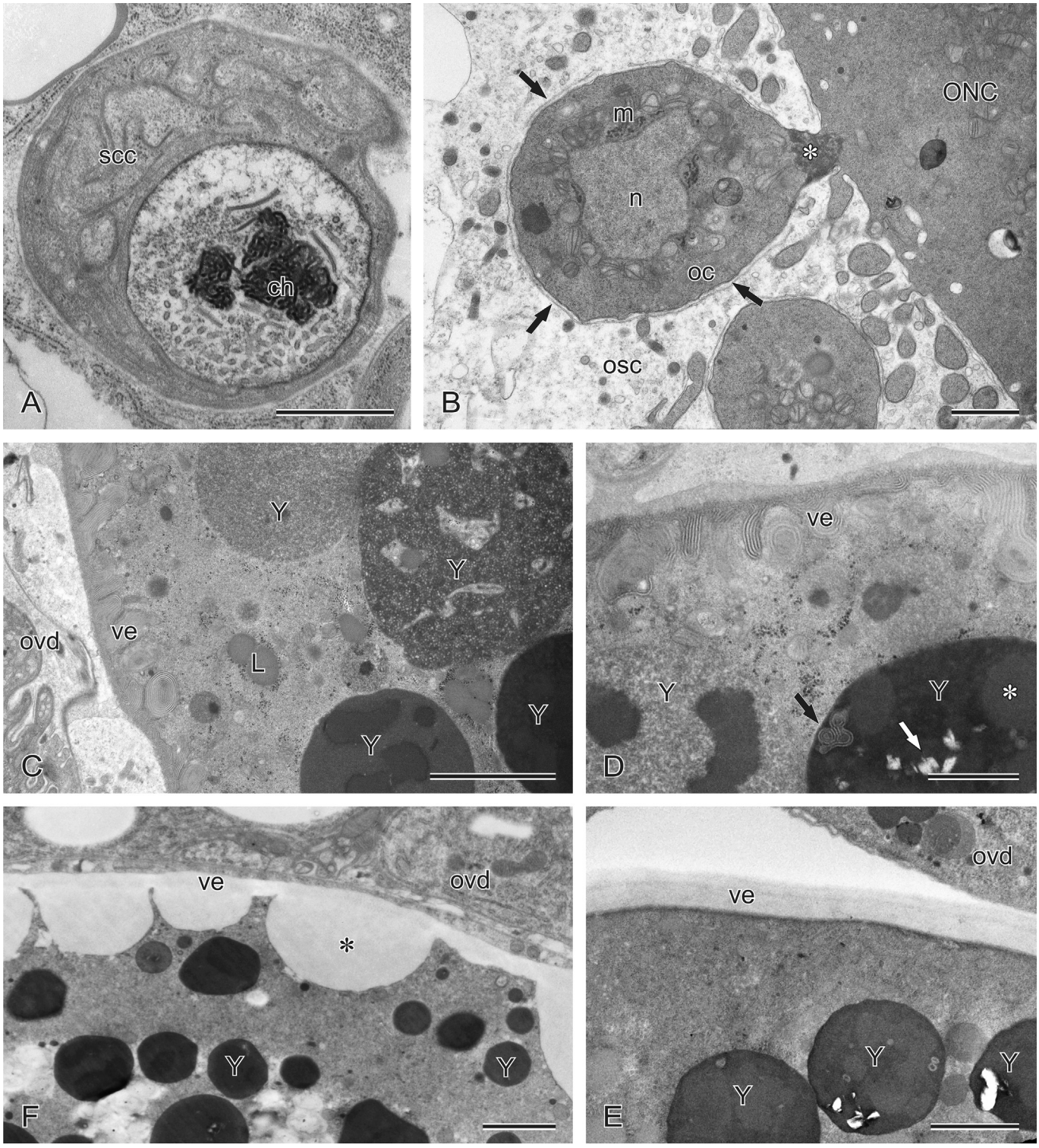

Fig. 6. Sperm-conveying cord in *Proctophyllodes fuchsi* (A), fragment of the ovary (B) and oviductal vitellogenic oocytes (C–E) in *Chaetodactylus osmiae*, and oviductal vitellogenic oocyte in *Falculifer rostratus* (F). (A) Cross-sectioned sperm-conveying cord (scc) containing spermatozoon with chromatin threads (ch). (B) Early oocyte (oc) with mitochondria (m) surrounding nucleus (n), which is connected via a bridge (asterisk) to the ONC. Note many protrusions of the ONC hosted in the ovarian stroma cell (osc). Oocytes are surrounded by ER cisterna of stroma cell (arrows). (C) Periphery of the vitellogenic oocyte containing yolk spheres (Y) in different stages of maturation, lipid droplets (L) and vitelline envelope (ve). (D) Higher magnification of the vitelline envelope (ve) showing a lamellated structure. Note the different appearance of two yolk spheres (Y); the left one is less mature and contains only several condensed cores in flocculent material, whereas in the more developed, dense right sphere there are paracrystalline cores (asterisk), electron-lucent inclusions (white arrow) and meandering structures (black arrow). (E) Bilayered vitelline envelope (ve) on an oviductal oocyte more developed than those in (C) and (D). Note that the vitelline envelope material is homogenous rather than lamellated and yolk spheres (Y) are well developed. (F) A vitellogenic oviductal oocyte in *Falculifer* has a well developed roughly spherical yolk (Y), lenticular extensions of vitelline space (asterisk), and very poorly contrasted vitelline envelope (ve). ovd – oviduct wall. Scale bars: 1 μm in (A); 2 μm in (B, D-F); 5 μm in (C).

The ONC (Figs 3 and 4A–C, E) is a very large cell (diameter from ca. 20 μm in *Falculifer* and *Pseudolichus,* 25–30 μm in *Sarcoptes*, *Scutulanyssus* and *Grallolichus*, 30–35 μm in *Canestrinia*, *Glycyphagus*, *Notoedres,* 40 μm in *Pterolichus*, up to 50 μm in *Histiostoma, Psoroptes* and *Dermatophagoides*) (Desch, 2001; Liana, 2004; Lekimme et al., 2005) containing an elaborated nucleus with a number of prominent nucleoli. In most species the ONC has been described as multinucleate (Pyroglyphidae: *D. farinae, Dermatophagoides pteronyssinus* – Walzl, 1992) or mononucleate with a multilobular nucleus (Acaridae: *A. siro* – Witaliński et al., 1990), but recent studies with serial sectioning following 3-D reconstruction performed on representatives of three families (Schwaha et al., 2008): *S. berlesei* (Acaridae), *G. domesticus* (Glycyphagidae), *Chorioptes bovis* and *Otodectes cynotis* (Psoroptidae) led to the conclusion that in all Astigmata in ONCs only one extensively branched nucleus is present. DNA-specific fluorescence after DAPI staining suggests polyploidy (Fig. 3D). The nuclear envelope forms many concavities rich in nuclear pore complexes with frequently adhering fine granular or flocculent nuage material. The rest of the cytoplasm is packed with free ribosomes; it also contains mitochondria and scarce ER and profiles of Golgi bodies. Lysosome-like bodies with non-homogenous content (*H. feroniarum*: Witaliński et al., 2014) as well as crystalline inclusions (*S. scabiei*: Desch, 2001) can also be found.

Eccentrically located ONCs are partly covered by oogonia and previtellogenic oocytes distributed usually in one to several layers; in this area the plasmalemma of the ONC can form many short protrusions described, e.g. in *S. scabiei* (Desch, 2001), as microvilli. In *G. domesticus* and *C. osmiae* the ONC also forms irregular, sometimes terminally expanded protrusions, penetrating the ovarian stroma cells at some distance (Figs. 5C and 6B). Oogonia and oocytes are connected with the ONC through conspicuous intercellular bridges (Figs. 4A, C, 5A, B and 6B). In general, intercellular bridges in all Astigmata except Histiostomatidae are funnel-shaped and filled with an electron-dense granular material (Fig. 6B) (Witaliński et al., 1990; Lekimme et al., 2005; Schwaha et al., 2008; Florek and Witaliński, 2010b; Klien and Walzl, 2010). The bridge opening at the ONC side is ca. 6 μm wide and is smaller than at the oocyte side. Oogonia and small, young oocytes have elongated and much thinner bridges, but their diameter increases with the growth of the cells. Recent preliminary studies (Florek and Witaliński, 2010b) indicated that in histiostomatid mites (*H. feroniarum*) a second, unusual type of intercellular bridge is present (Figs. 3A and 4B). This bridge is practically two-dimensional and very large in advanced previtellogenic oocytes (10 μm or more) but its lumen is crossed with a thin diaphragm of electron dense material, hence it has been termed a diaphragm-crossed bridge. At higher magnification, the dense material is arranged in a rectangular lattice. The same was observed in another undescribed *Histiostoma* species (Witaliński et al., 2014). In another histiostomatid mite, *Bonomoia opuntiae*, the bridge has somewhat intermediate structure since the diaphragm dense material is thickened in the center of the bridge lumen (preliminary observations).

Oocytes entering meiotic prophase are small roundish cells with a relatively large spherical nucleus in which synaptonemal complexes may be visible (Witaliński et al., 2014). After the first meiotic division, the oocytes start to grow at previtellogenic phase. In *S. berlesei*, meiosis is completed much later in oocytes moving along the second, backward-curved part of the oviduct, whereas the first cleavage into two blastomeres occurs at the beginning of the third, forward-curved part of the oviduct (Walzl et al., 2004).

Detailed studies on the architecture of the ovary and oocyte pathways within the ovary during their development are absent, but light-microscopical observations of semithin sections suggest that previtellogenic oogenesis occurs generally around the ONC towards the pole where the oviduct emerges. Thus, in most cases oogonia and early previtellogenic oocytes are distributed dorsally or dorso-laterally, whereas the oocytes entering vitellogenesis are distributed ventrally or ventro-laterally in the ovary, i.e. close to the oviduct entrance.

4.1.2. Vitellogenesis

Vitellogenesis in Astigmata occurs in oviductal rather than ovarian oocytes (Fig. 4C–G) (Witaliński, 1995; Walzl et al., 2004), but this process is known only superficially, since no investigations have dealt with the subject and data are only available from papers focused on other aims (Walzl et al., 2004; Lekimme et al., 2005; Witaliński et al., 2014). The nucleus in early vitellogenic oocytes is similar to that in previtellogenic ones but its outline is irregular (Fig. 4E); cytoplasmic organelles accumulate mainly at the oocyte periphery, whereas the rest of the cytoplasm is filled with two types of inclusions: electron-dense protein yolk spheres and electron-lucent inclusions, which are lipid droplets (Fig. 6C–F). The protein yolk spheres are usually several times larger than the lipid droplets; in *Psoroptes* (Lekimme et al., 2005) their size is 8 μm and 0.9 μm, respectively. Peripherally located protein yolk spheres are small, ca. 1.6 μm in *Histiostoma* and 1.0 in *Sancassania*. Much larger protein yolk spheres, ca. 5 μm in *Histiostoma* and up to 15 μm in *Sancassania,* are packed more centrally in the egg. Large spheres contain several darker roughly spherical cores of crystalline appearance. Lipid spheres comprise small lipid droplets (0.5–0.6 μm) grouped between protein yolk and sometimes surrounding areas of moderate density (Witaliński et al., 2014).

The origin of yolk protein (vitellogenin, Vg) varies among mite groups. In mites possessing an arachnid-type ovary (i.e. a hollow, tubular ovary with oocytes protruding outside on stalks and covered with basement membrane only), as in ticks and some Parasitengonina (for further references see Evans, 1992; Alberti and Coons, 1999; Coons and Alberti, 1999), the Vg is either produced by the oocyte itself or, rather, derived from external sources. Fat bodies, midgut cells, and specialized subepidermal cells have been proposed as external sources (for further discussion and references see: Cabrera et al., 2009). Externally produced Vgs are supplied through the hemolymph and absorbed via pinocytosis into oocytes. In many mites, however, the ovary is a compact structure and more or less evidently of nutrimental type (e.g. higher Gamasina: Arctacarina – Alberti and Krantz, 2007; Parasitina – Alberti et al., 1999; Dermanyssina – Alberti and Zeck-Kapp, 1986; Di Palma and Alberti, 2001; Nuzzaci et al., 2001; Di Palma et al., 2012; or some Parasitengonina: Erythraeidae – Witte, 1975). In such cases, the oocytes are supplied by nutritive cords from nurse cells with mitochondria, ribosomes and other cytoplasmic components (Steiner et al., 1995), as well as ribonucleoproteins involved in oocyte growth, but Vgs are synthetized and absorbed from hemolymph as above. In Oribatida, nutritive cells/tissues are absent in the ovary (for further references see Liana and Witaliński, 2012). In Astigmata, despite their close evolutionary relations to Oribatida, the ovaries are of nutrimental type, but the ONC seems to be a source of ribosome/ribosome subunits and probably mRNA for Vg authosynthesis rather than of Vg itself, since 1) cytoplasm of previtellogenic oocytes is highly saturated with free ribosomes (e.g. Fig. 5), 2) vitellogenesis starts and progresses in oocytes which are no longer connected via bridges with the ONC, and 3) when oocytes are transported along oviducts, they do not show any signs of intensive pinocytotic uptake; moreover, they are coated with a vitelline envelope (VE) transformed later into an impermeable chorion which can effectively block uptake from the hemolymph. It should be

noted, however, that newly formed VE in *S. berlesei* is lamellated and contains pores at regular intervals; it was suggested (Walzl et al., 2004) that yolk or yolk precursors can be transported from the oviduct wall into the egg via these pores. A similar lamellated VE also grows on oviductal oocytes in *C. osmiae* (Fig. 6C and D).

The molecular foundations of Vgs and their genes in Astgmata are, as in other mites, fragmentary and are known in only a few species (*Blomia tropicalis*, *D. farinae, D. pteronyssinus, G. domesticus, S. scabiei* and *Suidasia medanensis*) (Cabrera et al., 2009). Equally unexplored is the regulation of vitellogenesis and, in particular, the identification and physiological role of ecdysteroids and juvenile hormon (JH) in endocrine regulation of vitellogenesis. In mites, ecdysteroids such as ecdysone, 20E, 2-deoxyecdysone, and makisterone A have been identified in the gamasid mites, *Dermanyssus gallinae* (Chambers et al., 1996) and *Varroa jacobsoni* (Feldlaufer and Hartfelder, 1997), and an astigmatan, *Tyrophagus putrescentiae* (Sakagami et al., 1992). Because JH and its analogs have a major role in influencing oogenesis/vitellogenesis in most insects, investigations aimed at finding these substances in mites have been conducted. Only farnesol, a precursor of JH, was identified in deutonymphs of *Tetranychus urticae* (Regev and Cone, 1975, 1976). However, farnesol is present in plants and can be sequestered from food by females which may use this substance as a sex attractant. On the other hand it was shown that exogenous farnesol increase oviposition.

In contrast, many studies explored the effects of exogenous JH and its analogs or anti-JHs on mite reproduction (see Cabrera et al., 2009: Table 2), but in the case of Astigmata there was either no effect (farnesol, JH analogs: methoprene and pyriproxyfen on *T. putrescentiae*) or the effect was negative (JH analogs: fenoxycarb, hydroprene and methoprene on *D. farinae*; ecdysone analog: halofenozide on *T. putrescentiae*). The only positive effect was shown by the JH analog, fenoxycarb, on female reproduction in *A. siro*. In conclusion, studies with JH precursors and anti-JHs were equivocal in establishing that mites have insect JH or they use it to regulate reproduction; thus a new concept for the regulation of female reproduction in mites was proposed in which ecdysteroids instead of JHs play the main role in stimulation of Vg gene expression in the fat body and midgut in ticks, or the midgut and ovaries in other mites (Cabrera et al., 2009).

4.1.3. Egg envelopes

Eggs of Astigmata are protected by envelopes of complex origin (Witaliński, 1993). First, an early vitellogenic oocyte entering the oviduct starts to secret a VE, which is therefore of primary origin. TEM studies on VE structure and formation indicated that at the beginning the VE material is either lamellated or coarse-fibrillar (*A. siro*, *Tyrophagus, S. berlesei* – Acaridae; Witaliński, 1993; Walzl et al., 2004), but is not penetrated by oocyte microvilli as suggested by Reger (1977) for *Caloglyphus anomalus* (=*Sancassania anomala*) (Acaridae), a species with a VE of the same appearance in TEM as *A. siro* (Witaliński, 1993) and *C. osmiae* (Fig. 6C and D). In *Psoroptes* spp. (Lekimme et al., 2005), the VE is homogenous and electron-lucent, and its thickness is 0.2–0.3 μm. An electron-lucent VE also appears on oocytes in *F. rostratus*; the oocyte, as in other psoroptid mites, forms many deep concavities (Figs. 4D, F and 6F). The early VE in *Histiostoma* sp. has a variable thickness (0.6–1.5 μm) and its material is electron-dense, but contains ellypsoidal lucent spaces (Witaliński et al., 2014). In *S. berlesei* the lamellated VE is 1 μm thick (Walzl et al., 2004).

As observed in several astigmatans, the thickness of the VE changes: at the beginning of VE formation its thickness increases, but decreases later when the VE lamellae or fibrils disappear, leading to a homogenous VE (see Fig. 6D and E). Interestingly, the disappearance of VE substructure is concomitant with up to a twofold decrease in VE thickness and occurs when the eggs are passing through the distal third portion of the oviduct. Its wall is thick and possibly contains secretory cells. Secretion was not evidenced, but secretory activity was suggested to be a source of VE modifications in structure and properties (Witaliński, 1993). A modified, homogenous VE is 0.3–0.4 and 0.7 μm thick in *A. siro* and *T. perniciosus*, respectively, and in *A. siro* it was named the chorion since it was the only layer enveloping a deposited egg. In other species, additional, exochorional material can be deposited on the egg chorion (which therefore is termed the endochorion) prior to laying. In the distal portion of oviducts in *T. perniciosus* and putatively *Tyrophagus longior*, an exochorion of three types is secreted: dense patches, granules, and locular chambers. In *Aleuroglyphus ovatus*, tiny spherical patches were found instead of locular chambers. In psoroptid mites *S. scabiei, N. cati,* and *F. rostratus*, a VE of flocculent appearance also transforms into a homogeneous chorion.

Chorional glands in sarcoptid mites release exochorion material on the egg surface and form a vesicular monolayer (Witaliński, 1993). In *F. rostratus*, the chorional gland (Fig. 4D and F)produces a substance which is used to glue the egg on the feather barb of its host (the pigeon). It seems likely that the function of adhesive exochorion material is mainly egg fixation to substratum (*Sarcoptes*, *Notoedres*, *Falculifer*, pterolichoid feather mites – Dubinin, 1953), but additional functions were also proposed, e.g. limited water loss from eggs due to locular chambers in *Tyrophagus* (Witaliński, 1993).

4.2. The male reproductive system

Male reproductive systems in Astigmata were studied on several occasions by light-microscopy (Michael, 1901; Rohde and Oemick, 1967; Prasse, 1968; Heinemann and Hughes, 1970; Kuo and Nesbitt, 1970; Vijayambika and John, 1975; Baker and Krantz, 1985; Witaliński and Walzl, 1995) and ultrastructurally (Witaliński et al., 1990; Walzl, 1992; Lekimme et al., 2005) and are more variable in organization compared to female reproductive systems. They comprise paired spherical or ellypsoidal testes located usually symmetrically in the rear part of the idiosoma, two deferent ducts (*vasa deferentia*), and one, two or no accessory glands (e.g. Fig. 2E and F). The proximal part of deferent ducts serves as a sperm reservoir, while the distal one has a glandular character (Witaliński et al., 1990). In males of analgesoids *A. passerinus* (Analgidae) and *Trouessartia appendiculata* (Trouessartiidae), deferent ducts empty into vesicular structure described by Dubinin (1951) as a seminal vesicle; however, further studies seem necessary to clarify its structure and function. Both deferent ducts merge together with duct(s) of accessory gland(s), if present, to form a short unpaired deferent duct passing into an ectodermal, cuticle-lined ejaculatory duct (*ductus ejaculatorius*). In *Pterodectes* (Proctophyllodidae) the ejaculatory duct is an extremely complicated structure functioning during insemination as a sperm pump (Popp, 1967; Alberti and Coons, 1999). The ejaculatory duct enters the aedeagus to terminate at its apex.

In acarid mite *Caloglyphus* (=*Sancassania*) *berlesei*, the testes are situated asymmetrically: the left testis is located dorsally whereas the right one is located ventrally as a result of the occupation of the left side of the body by a very large accessory gland (Witaliński and Walzl, 1995). In *Lardoglyphus konoi* (Acaroidea: Lardoglyphidae), one testis is in front of the other, and two different accessory glands (one of them named a chambered organ) are present (Vijayambika and John, 1975). In the hemisarcoptoid mite *C. osmiae* two testes – each with its own germarium – adhere very tightly to one another filling the right side of the idiosoma, whereas the left side is occupied by a large accessory gland which opens into the left deferent duct (Fig. 7B). In the glycyphagoid *G. domesticus* two testes

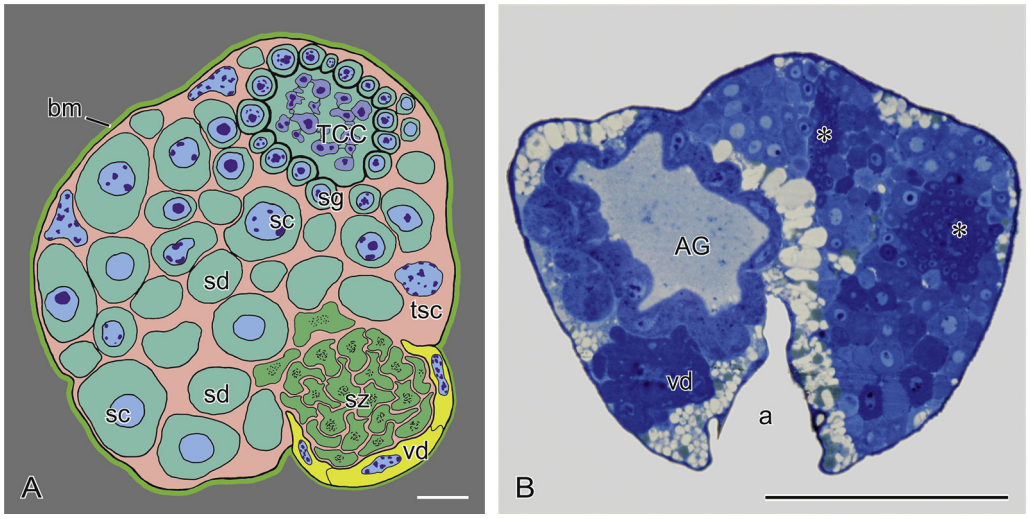

Fig. 7. (A)Testis organization as visible in cross sections of male mites in case the germarium contains a testicular central cell (TCC) (e.g. Acaridae). The TCC with ramified nucleus is tightly surrounded by spermatogonia (sg) whereas spermatocytes (sc) and spermatids (sd) in various developmental stages are spread in testicular stroma cells (tsc). The entrance of deferent duct (vd) collects maturing sperm cells (sz). The basement membrane (bm) of testicular stroma cells surrounds the testis. (B) Semithin cross section of *Chaetodactylus osmiae* male showing two germaria (asterisks) in closely located testes at right side and a large accessory gland (AG) which occupies the left side of the mite body. a – anal opening, vd – deferent duct. Scale bars: 10 μm in (A); 100 μm in (B).

are displaced symmetrically (Fig. 8A), but the left one is shorter as a large accessory gland is located anteriorly (Fig. 8B). In *Psoroptes ovis* (Psoroptidae) (Fig. 2E and F), paired testes are secondarily fused at their proximal parts, thus being actually unpaired (Lekimme et al., 2005). Similarly, in *N. cati* (Sarcoptidae), testes are interconnected by a narrow bridge which is filled with an electron-dense, flocculent material (Witaliński, 1988). In *D. farinae* and *D. pteronyssinus* (Pyroglyphidae), the testis is unpaired (Walzl, 1992). In the sarcoptid mites *S. scabiei* and *N. cati,* testes are situated anteriorly to the aedeagus due to its posterior shifting while short, quickly merging deferent ducts run postero-ventrally rather than antero-ventrally (as in most other astigmatans), to empty into the beginning of the ejaculatory duct (Witaliński and Walzl, 1995).

The aedeagus is a sclerotized organ located midventrally, protruded by hydrostatic pressure and retracted by muscles attached to sclerites in the genital atrium (Prasse, 1970). Its tip is shaped to fit the female bursa copulatrix opening; in *Proctophyllodes* males, the aedeagus is extremely long extending far behind the body of the male (OConnor, 2009).

4.2.1. The testis

Testes in astigmatans have usually been briefly described in conjunction with studies on spermatogenesis and sperm structure (Alberti, 1980; Witaliński et al., 1986; Witaliński and Afzelius, 1987; Witaliński, 1988; Witaliński et al., 1990; Walzl, 1992; Florek and Witaliński, 2010a; Lekimme et al., 2005). They are rather compact organs (Fig. 7A), delimited by a thin amorphous layer (Vijayambika and John, 1975) resembling a basal lamina in the light microscope. The membrane surrounds a few somatic cells in which germ cells at different stages are embedded (Witaliński et al., 1990; Walzl, 1992). In this review, such somatic cells are termed the testicular stroma cells. In some species (*D. columbae, F. rostratus*), stroma cells are multinucleate and perhaps syncytial (Fig. 9A and B). In *S. scabiei* two other kinds of somatic cells were found: distal cells and muscle cells (Witaliński and Afzelius, 1987).

The germinal part (germarium) of the testis can be located dorsally or dorsolaterally (in *S. scabiei* and *N. cati* – Witaliński and Walzl, 1995; *C. lactis* – Florek and Witaliński, 2010a), but if the testis is elongated then the dorsally located germarium may be found either in the anterior or posterior part of the gonad, as in *G. domesticus* (Witaliński and Walzl, 1995) (Fig. 8A) and *A. siro* (Witaliński et al., 1990), respectively. In *C. osmiae* the germaria are located less regularly but rather adaxially in both testes adhering closely to each other as mentioned above.

The germarium is composed of a compact group of early germ cells, spermatogonia, adhering tightly to one another (Fig. 8C and D) and to the so-called testicular central cell (TCC), if such a cell is present in the adult gonad. The TCC (Fig. 8C) has been reported in *A. siro* (Witaliński et al., 1990), *Sancassania* (=*Caloglyphus*) *berlesei* and *S.* (=*Caloglyphus*) *michaeli* (Prasse, 1968), *Rhizoglyphus robini* (Baker and Krantz, 1985) and *H. feroniarum* (Florek and Witaliński, 2010a), but is absent in many other species: *D. pteronyssinus, D. farinae* (Walzl, 1992), *G. domesticus, S. scabiei, N. cati* (Witaliński and Walzl, 1995), *C. lactis* and *F. rostratus* (Florek and Witaliński, 2010a). The TCCs are of special interest since their origin – germinal or somatic – and function were for a long time enigmatic. The very similar placement of ONCs and TCCs in gonads suggested a germinal origin and, moreover, a nutritive function. However, intercellular bridges connecting spermatogonia with the TCC were not observed, thus the question remained open. Studies performed recently on gonad development in *Histiostoma* provided evidence that ONCs and TCCs belong to the germinal cell line (Witaliński et al., 2014), because both are connected by bridges with surrounding gonial cells in a quite similar way. The nutritive role of TCCs is rather doubtful; instead, it was postulated (Florek and Witaliński, 2010a) that the TCC can "suppress and/or drive the proliferation of adjacent spermatogonia" (for further discussion see Florek and Witaliński, 2010a). Indeed, growing and subsequently proliferating spermatogonia lose tight contact with the TCC, as was observed in *H. feroniarum* (unpublished) and *A. siro* (Fig. 8C), or separate from a compact mass of germarial earliest spermatogonia in species in which the TCCs in testes are absent (Fig. 8D).

The deferent duct entrance is located opposite to the germarium, usually in the ventral area of the testis (Fig. 7A). The entrance of the deferent duct and its vicinity are packed with sperm cells (Figs. 8A and 9A). Spermatocytes and spermatids disperse after leaving the germarium which disrupts the sequential distribution of spermatogenesis stages (Fig. 9A). They disperse singly or in small clonal groups which do not form wall-encapsulated cysts; rather, they are hosted within large, sometimes multinucleate somatic

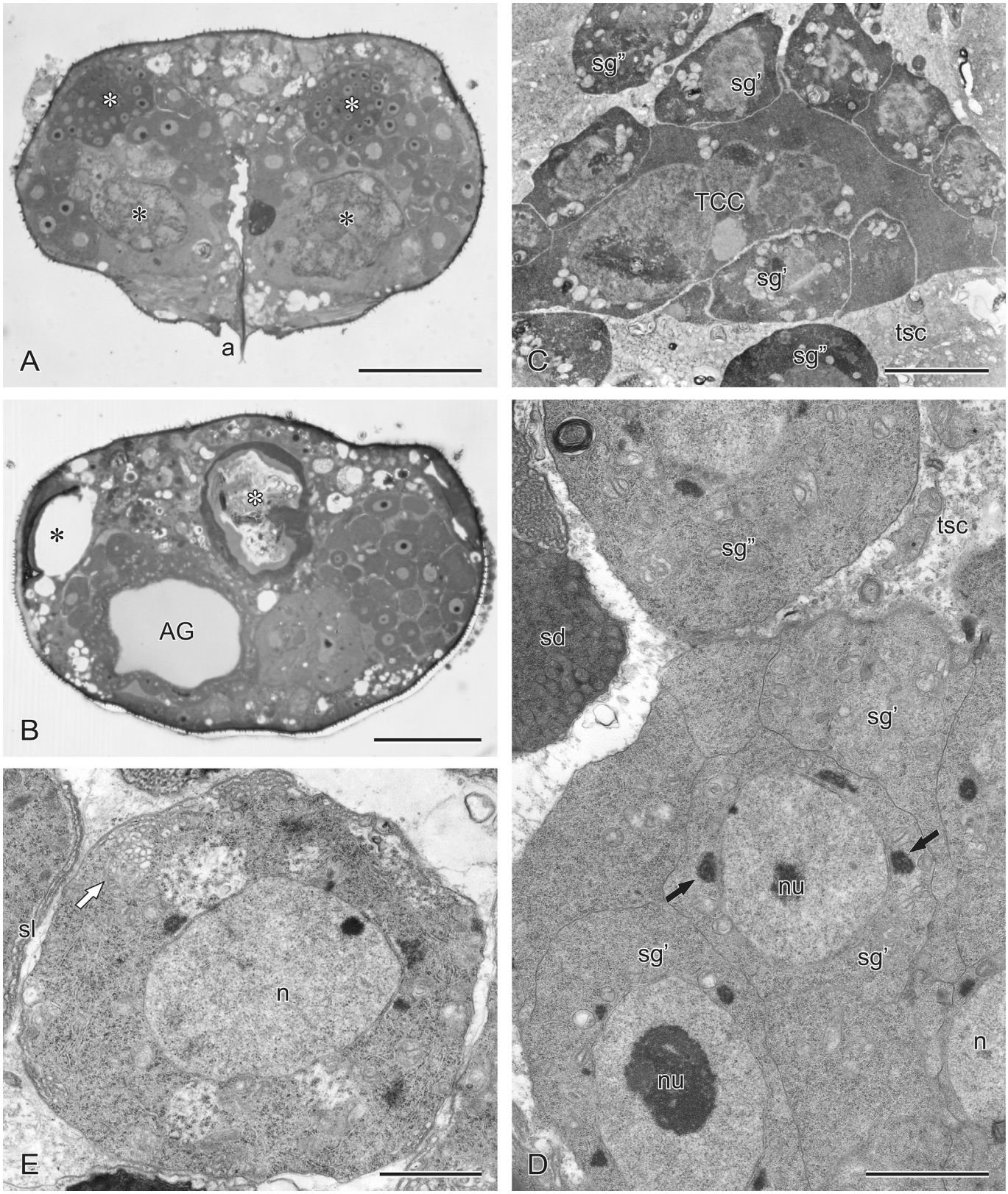

Fig. 8. Two semithin sections of *Glycyphagus domesticus* male in light microscope (A, B) and details of the testis in TEM, in *Acarus siro* (C) and *Carpoglyphus lactis* (D, E). (A) Section at anal opening (a) level shows two symmetrically distributed testes, each with dorsally located germarium (white asterisks) and ventrally visible spermatozoa (black asterisks) within or close to the entrance of a deferent duct. (B) section as in (A) but more anteriorly. Anal opening is absent, right testis still occurs, but the left one is replaced by a large accessory gland (AG). Lumen of opisthonotal gland and alimentary tract are indicated by black and white asterisks, respectively. (C) Germarium containing the testicular central cell (TCC) surrounded tightly by early spermatogonia (sg'). Proliferating spermatogonia (sg'') are separated from the TCC. (D) Early spermatogonia (sg') in germarium adhere tightly to each other, whereas proliferating ones (sg'') are distant. Note early spermatogonia nuclei with prominent central nucleoli (nu) and nuage material adhering to nuclear envelope (arrows). (E) Spermatocyte showing formation of spongy layer with participation of Golgi body (arrow). In a more advanced spermatocyte a spongy layer (sl) is formed. n – nucleus, sd – spermatid, tsc – testicular stroma cell. Scale bars: 50 μm in (A, B); 5 μm in (C); 2 μm in (D, E).

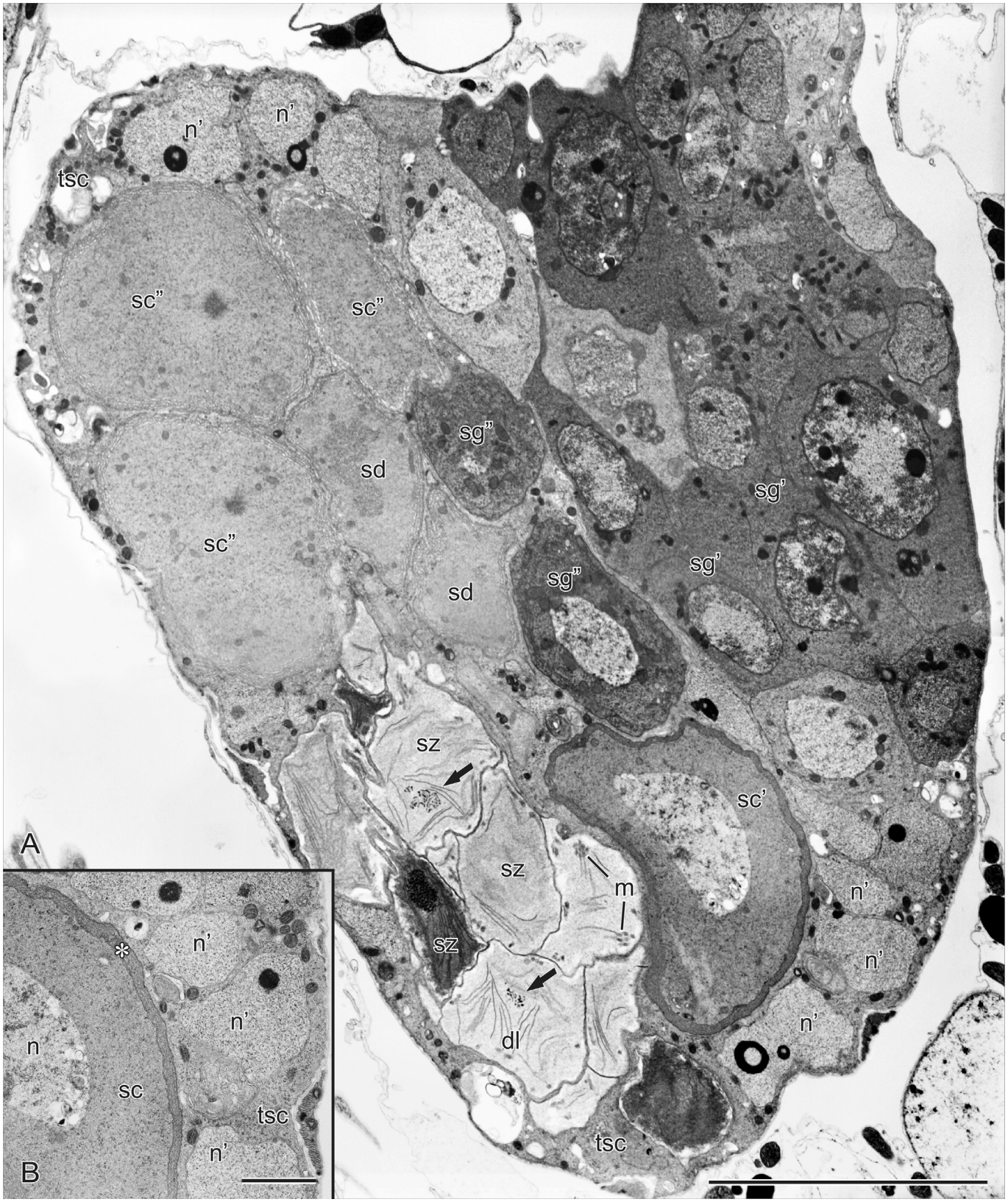

Fig. 9. Section through the testis in *Falculifer rostratus* in TEM. (A) Early spermatogonia (sg') form the germarium and are electron-dense; proliferating spermatogonia (sg") are located at the germarium periphery whereas a spermatocyte (sc') before division is less dense and enveloped in a thick spongy layer. Dividing spermatocytes (sc") and spermatids (sd) show lower density and their nuclei are absent. A spongy layer still occurs. Spermatozoa (sz) are electron-lucent and contain dense lamellae (dl), grouped mitochondria (m), and chromatin threads (arrows). Somatictesticular stroma cells (tsc) are multinucleate and their nuclei (n') are peripherally distributed. (B) Fragment of the testis. Spermatocyte (sc) containing a nucleus (n) is covered by a spongy layer (asterisk). Several nuclei (n') of a testicular stroma cell (tsc) are marked. Scale bars: 10 μm in (A); 2 μm in (B).

cells filling the testis, i.e. the testicular stroma cells (Figs. 8C–E and 9A, B).

4.2.2. Spermatogenesis

As mentioned above, the earliest spermatogonia located in the germarium adhere to the TCC or, if a TCC is absent, they adhere to the other spermatogonia so tightly that their borders can be detected only by TEM (Fig. 8C and D). A relatively large spermatogonial nucleus contains a prominent central nucleolus (Fig. 8D). The nuclear envelope shows shallow concavities with adhering nuage material. Mitochondria are distributed close to the nucleus. Spermatogonia located at the periphery of the germarium are larger and make contact with the germarium one-sidedly (Fig. 8D), eventually to separate and enter the spermatocyte stage.

Early spermatocytes (Fig. 8E) are singular spherical cells with a roundish, "empty" nucleus devoid of condensed chromatin, but usually with a nucleolus located peripherally. Nuage material is still present. As the spermatocyte grows, the peripheral cytoplasm hosts many mitochondria and Golgi bodies; the latter participate in the formation of a characteristic superficial spongy layer of anastomosing membranes/cisternae covering the cell (Figs. 8E, 9B and 10A). The contribution of Golgi bodies to spongy layer formation was observed in many species (Witaliński et al., 1986, 1990; Liana, 2004; Florek and Witaliński, 2010a), nevertheless, subplasmalemmal cisternae of ER were also proposed to be a source of spongy layer membranes in Astigmata spermatocytes (Witaliński and Afzelius, 1987; Lekimme et al., 2005).

In general, growing spermatocytes undergo division (Fig. 10B and C), however, division is doubtful in some astigmatic species (see below). During division neither the nuclear envelope nor condensed chromatin with synaptonemal complexes is visible. This suggests that normal meiosis may be absent. Daughter cells remain interconnected by bridges delimited by unthickened cisterns of the spongy layer, thus typical contractile rings lined with electron-dense material typical for conventional intercellular bridges or ring canals (permanent intercellular bridges in gametogenesis) are likely absent (Florek and Witaliński, 2010a). The number of interconnected spermatids is no more than four, since in most species, sectioned groups of spermatids contain two (*C. lactis* – Florek and Witaliński, 2010a) or three to four (*A. siro, T. putrescentiae* – Witaliński et al., 1986, *D. columbae* – Fig. 10C) cells in section, whereas in some species, e.g. in *G. domesticus* and *C. osmiae*, spermatocytes and spermatids are singular. Moreover, the size of spermatids is only somewhat smaller than that of spermatocytes, decreasing progressively. In such cases, male germ cells differentiate, but evidently do not divide.

During spermiogenesis many unusual changes occur. Mitochondria in early spermatids can be located peripherally beneath the spongy layer (e.g. in *C. lactis* – Florek and Witaliński, 2010a); later, in many species they transform into more or less modified mitochondrial derivatives, sometimes vesicular and difficult to distinguish, as in *A. siro, T. putrescentiae* (Witaliński et al., 1986, 1990), *H. feroniarum* (Witaliński et al., 2014), *D. columbae* (Fig. 10B and C), or *P. fuchsi* (Fig. 10E). In some cases, however, mitochondrial morphology is altered moderately (e.g. *S. scabiei* and *N. cati* – Witaliński and Afzelius, 1987; Witaliński, 1988; *C. osmiae* – Fig. 11B), or only slightly, as in *G. domesticus* (Fig. 11D and E) or *F. rostratus* (Liana and Witaliński, 2005). In some species, mitochondria have a tendency to aggregate forming large assemblages, as in *D. pteronyssinus* and *D. farinae* (Walzl, 1992); in *P. obtusus* many rod-shaped mitochondria aggregate end-to-end and side-by-side forming bundles meandering within the cell (Liana and Witaliński, 2005).

As spermiogenesis progresses, cisterns of the spongy layer can aggregate to form a spherical spongy body (Fig. 11E, inset) (Florek and Witaliński, 2010a); cisterns of the spongy layer sometimes participate in the formation of very conspicuous structures (Fig. 11A and C). In consequence, the spermatid is no longer covered by a spongy layer, but its plasmalemma seems to be thickened.

Chromatin appears in spermatid cytoplasm as progressively thickening threads. Electron-dense lamellae (most species) or tubules (*Sarcoptes* and *Notoedres*) derived from ER also occur. In some cases, a band of granular material (*C. sellnicki*) or many chains of small vesicles (*Scutulanyssus obscurus*) occur in the spermatids, but dense lamellae or tubules are absent (Liana, 2004).

4.2.3. Sperm structure

Several papers dealing with sperm structure in Astigmata have described their peculiar organization: *S. anomala* (=*C. anomalus*) (Reger, 1971), *A. siro* (Alberti, 1980; Witaliński et al., 1986, 1990), *T. putrescentiae* (Witaliński et al., 1986), *D. farinae* (Walzl, 1992), *Psoroptes equi* (Alberti, 1984), *S. scabiei* (Witaliński and Afzelius, 1987), and *N. cati* (Witaliński, 1988). In the only review dealing with sperm structure in Astigmata (Liana and Witaliński, 2005), the number of studied species was substantially enlarged to include *H. feroniarum, C. sellnicki, Glycyphagus* sp., *S. berlesei*, *P. obtusus*, *P. phasiani, G. proctogamus*, *F. rostratus*, *S. obscurus, Trouessartia minutipes* and *Myocoptes musculinus.* Quite recently, data on the ultrastructure of spermatozoa in *C. lactis* have become available (Florek and Witaliński, 2010a). Sperm structure in four other species, *C. osmiae* (Hemisarcoptoidea: Chaetodactylidae), *G. domesticus* (Glycyphagoidea: Glycyphagidae), *D. columbae* (Analgoidea: Analgidae), and *P. fuchsi* (Analgoidea, Proctophyllodidae), are described in this review and thereby increase the number of studied species to 23. Representatives of 8 superfamilies from 9 available for investigation were studied and only spermatozoon structure in Hypoderatoidea is still unknown.

Spermatozoa in astigmatic mites are multiform cells (Figs. 9A and 10D, E and 11B, D, E) making their dimensions difficult to precisely determine. As calculated from TEM micrographs, sperm cell size varies considerably from ca. 2 μm in *H. feroniarum,* 3–4 μm in *M. musculinus, N. cati* and *S. scabiei,* 5 μm in *G. proctogamus,* 6 μm in *A. siro,* 6–7 μm in *F. rostratus* and *D. farinae*, 7–8 μm in *Glycyphagus* sp., 8–10 μm in *P. phasiani*, 8–11 μm in *P. obtusus* and 12 μm in *T. minutipes,* but reaching ca. 18 μm in *C. sellnicki* and 22–27 μm in *S. obscurus* (Liana and Witaliński, 2005). Sperm diameter in the newly studied species *D. columbae* is ca. 1.4–2.9 μm, 2.9–4.5 μm in *P. fuchsi*, whereas in *C. osmiae* and *G. domesticus* spermatozoon size is ca. 4 × 11 μm and in 7 × 15 μm, respectively.

The most striking feature of sperm cells is the lack of a nucleus; instead, chromosomal material is visible as threads embedded directly in the cytoplasm and located more or less centrally in the cell (e.g. Alberti, 1980; Lekimme et al., 2005; Liana and Witaliński, 2005; Florek and Witaliński, 2010a). Chromatin has a star-like appearance in two of three genera in the Pterolichidae family, suggesting that several threads cross at some sites. The diameter of chromatin threads varies from 40 to 60 nm in most species up to 170–190 nm in *H. feroniarum.*

Other peculiarities of Astigmata sperm are electron-dense lamellae derived from flat ER cisternae which are present in spermatids. In *C. lactis* there is only one lamella partly surrounding the chromatin threads (Florek and Witaliński, 2010a). In the *Histiostoma* spermatozoon (Liana and Witaliński, 2005; Witaliński et al., 2014) two lamellae run parallel to each other and this tandem is located laterally to the chromatin threads. Two to several lamellae occur in the vicinity of chromatin threads in *Tyrophagus* (Witaliński et al., 1986). In several other species shorter or longer profiles of lamellae, frequently in parallel arrangement, are placed mostly around the chromatin (*Acarus*, *Glycyphagus*, *Dermatophagoides*, *Falculifer*, *Grallolichus*, *Psoroptes*, *Myocoptes*), whereas in *Pterolichus*

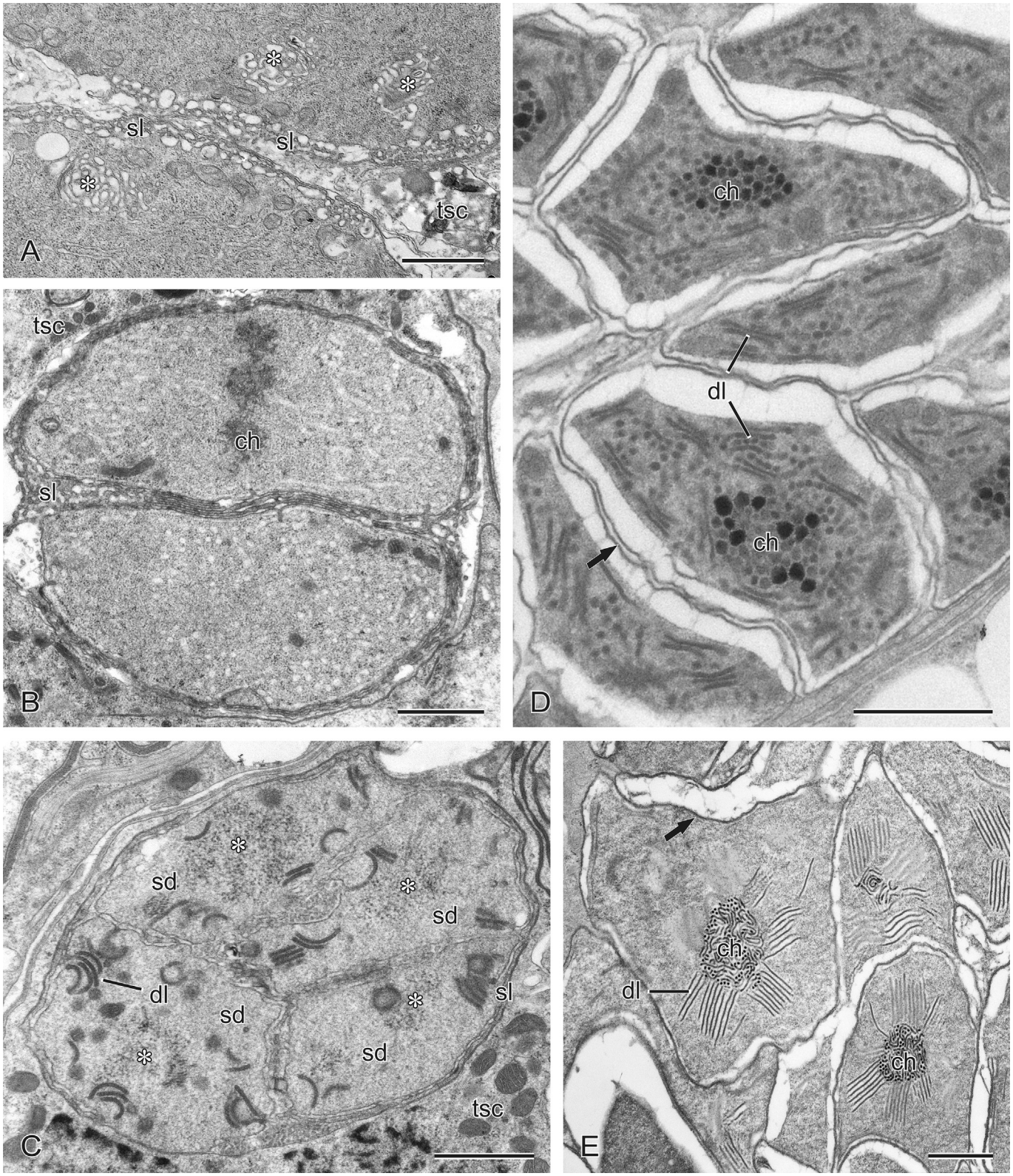

Fig. 10. Spermatogenesis and sperm structure in *Carpoglyphus lactis* (A), *Diplaegidia columbae* (B–D), and *Proctophyllodes fuchsi* (E), TEM. (A) Two spermatocytes with Golgi bodies (asterisks) contributing to spongy layer (sl) formation. (B) Spermatocyte division. In the upper cell condensed chromatin (ch) is visible. (C) Four spermatids (sd) with fine chromatin (asterisks) and short electron-dense lamellae (dl). Note that the spongy layer is thinner than in spermatocytes (B). (D) Spermatozoa showing granular chromatin (ch) and moderately electron-dense profiles (dl). A spongy layer is absent, but the sperm plasmalemma (arrow) is thickened. (E) Spermatozoa containing chromatin threads (ch) and electron-dense lamellae (dl). Thickened plasmalemma indicated by arrow. sl – spongy layer, tsc – testicular stroma cell. Scale bars: 1 μm in (A–E).

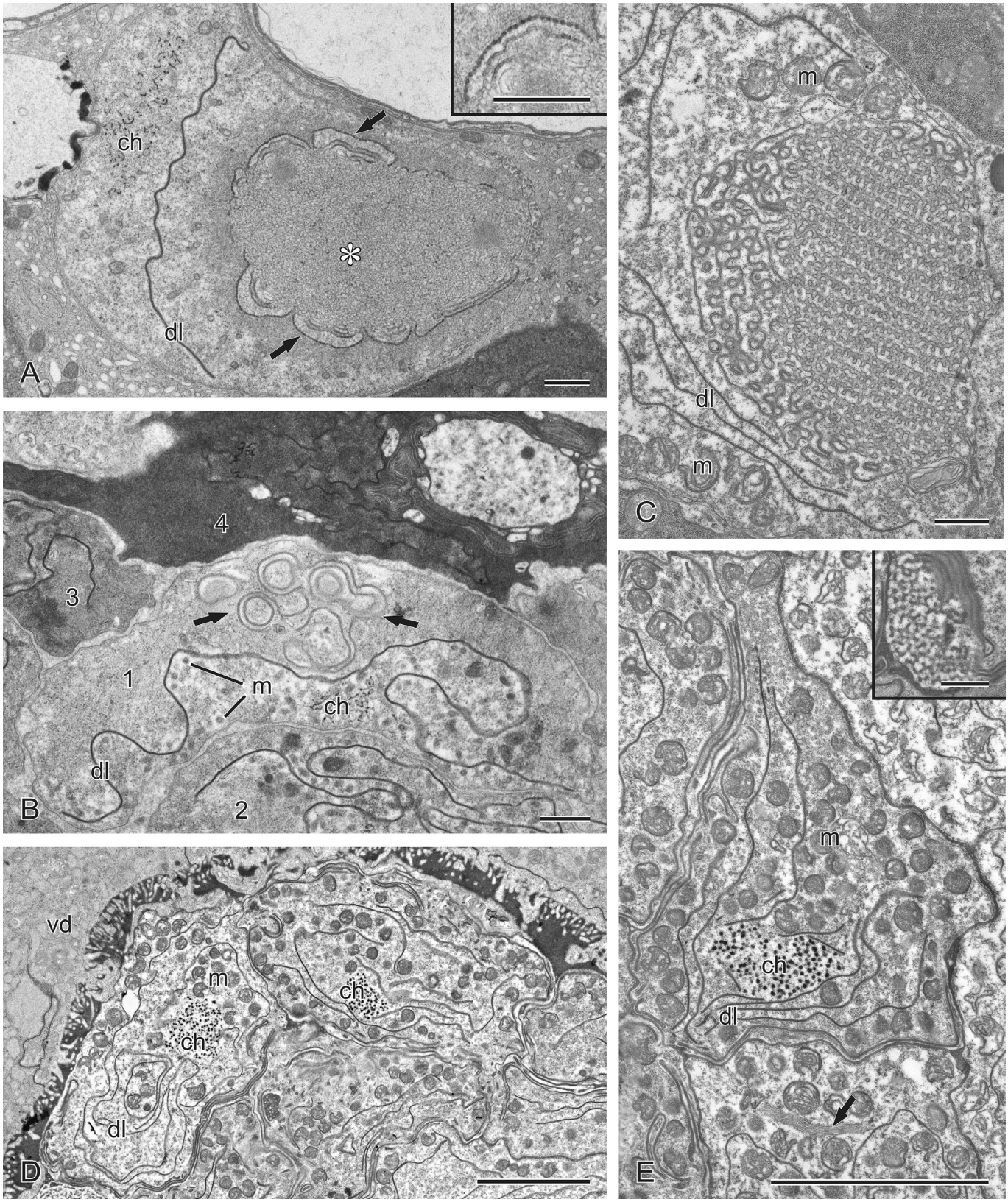

Fig. 11. Spermatogenesis and sperm structure in *Chaetodactylus osmiae* (A, B) and *Glycyphagus domesticus* (C–E), TEM. (A) Spermatid with chromatin (ch) and area of anastomosing membranes (asterisk) separated by electron-dense lamella (dl). Anastomosing membranes are surrounded by arcuate cisterns (arrows); their external membranes show periodic densities (inset). (B) Several sperm cells at different levels of condensation (1–4): less condensed spermatozoon (1) shows an electron-dense lamella (dl) which separates chromatin threads (ch) from circular profiles (arrows). Mitochondrial derivatives (m) are also present. (C) Spermatid fragment containing a conspicuous cisternal arrangement which later transforms into a spongy body of the spermatozoon. There are also electron-dense lamellae (dl) and mitochondria (m). (D) Several spermatozoa in a deferent duct (vd) containing chromatin (ch), dense lamellae (dl), and mitochondria (m). Material filling the deferent duct is highly electron-dense. (E) Spermatozoon at higher magnification showing chromatin (ch), dense lamellae (dl), and mitochondria. In the lower spermatozoon a fibrillar bundle (arrow) is present. Inset shows a spongy body. Scale bars: 1 μm in (A–C); 5 μm in (D, E); 1 μm in (E inset).

they are visible in between chromatin threads; in *Pseudolichus* lamellae are scarce and curved showing circular profiles. Spermatozoa in *Trouessartia* are packed with stacks of parallel and short lamellae. In contrast, lamellae are absent in *Canestrinia* and *Scutulanyssus* sperm; in the former species sperm contains a band of granular material, whereas the *Scutulanyssus* sperm cell is filled with branching chains of vesicles (Liana and Witaliński, 2005). In sarcoptid mites (*Notoedres* and *Sarcoptes*), ramifying electron-dense tubules distributed within the cell are visible in place of lamellae; these tubules originate during spermiogenesis from tubular profiles of ER rather than flat ER cisternae (Witaliński and Afzelius, 1987). *Psoroptes* sperm shows intermediate lamellar structures since spermatozoa contain many parallel lamellae organized in groups and electron-dense tubules which seem to grow out from the lamellae margins (Alberti, 1984; Liana and Witaliński, 2005).

As was mentioned earlier, mitochondria during spermiogenesis in Astigmata either persist – at least to some degree – in their normal structure, or they transform into mitochondrial derivatives of variable appearance and distribution within the cell. Nearly unmodified mitochondria have been found in sperm of *P. obtusus* (Liana and Witaliński, 2005), in which they form large assemblages with rod-shaped mitochondria aggregating end-to-end and side-by-side in a bundle meandering within the cell. Distinct and large assemblages of mitochondria are also present in *Dermatophagoides* sperm. In other studied species mitochondrial derivatives are more or less altered and their internal structures, especially cristae, are no longer discernible; such poorly visible vesicular structures occur, for instance, in *Acarus* and *Tyrophagus* sperm (Witaliński et al., 1986, 1990). Thus, in most cases the function of mitochondrial derivatives as energy (ATP) donors for spermatozoon movement seems unlikely; their deeply disintegrated structure is in accordance with the uniparental theory of maternal mitochondrial inheritance (e.g. Giles et al., 1980; Sutovsky et al., 1999).

Sperm structure in the currently studied species also shows some special characters. In *C. osmiae* (Fig. 11B) the spermatozoon contains only one meandering lamella which separates chromatin threads (40–45 nm thick) and circular profiles likely derived from spermatid arcuate cisterns present around a large spongy area (Fig. 11A). The latter is putatively a remnant of the spermatocyte superficial spongy layer. Spermatozoa in *G. domesticus* (Fig. 11D and E) contain many electron-dense lamellae surrounding chromatin threads (70–80 nm thick), as well as many globular mitochondria with frequent incisions. In the sperm cell of *P. fuchsi* (Fig. 10E) centrally located, tightly packed chromatin threads (27–33 nm thick) are surrounded by radially oriented groups of lamellae arranged in parallel. Most remarkable is the sperm of *D. columbae* (Fig. 10D), since this is the only case of astigmatic spermatozoon without chromatin threads; instead, there are many granules of variable size (90–125 nm) and density surrounded by moderately dense, elongated or circular profiles derived from a short electron-dense lamella visible in spermatids (Fig. 10C).

The acrosome is absent in sperm of Astigmata. This is the consequence of early syngamy, since sperm penetrates ovaries (Prasse, 1968; Witaliński et al., 1986; Witaliński, 1988) and encounters naked oocytes before the vitelline envelope/chorion is formed. Interestingly, spermatozoa found within the female show fine filaments regularly distributed under the cell membrane; their role in sperm motion have been suggested (Alberti, 1980; Witaliński et al., 1986).

4.2.4. Testicular somatic cells

Germinal cells in testis are embedded in a few somatic cells (Figs. 7A and 8C, D and 9), termed the testicular stroma cells in this review. The number of stroma cells is difficult to determine; their irregularly shaped nuclei located peripherally are scarcely visible, suggesting a low number of these cells in the testis (e.g. Sarcoptidae: *N. cati* – Witaliński, 1988). In some species, e.g. *C. lactis*, stroma cells are distinct and connected by adherent junctions, at least close to the testis-deferent duct transition (Florek and Witaliński, 2010a). However, in *F. rostratus* (Fig. 9) and *S. scabiei,* stroma cells (originally named the main somatic cells; Witaliński and Afzelius, 1987) contain many nuclei and their syncytial nature is possible. In the latter species, two additional kinds of somatic cells were found: the so-called distal somatic cells and muscle cells. The distal somatic cells are located close to the beginning of the deferent duct, thus may be considered as an element of the deferent duct wall. More interesting are muscle cells embedded in stroma cells, with contractile filaments containing appendages penetrating stroma cells and observable between germ cells in testis regions rather distant from the entrance of the deferent duct.

5. Conclusions and perspectives

Studies on the reproduction of Astigmata are fragmentary and focused mostly on some aspects of reproduction and reproductive behavior having implications in the evolution of reproductive strategies (Tilszer et al., 2006; Radwan, 2009) rather than gonad structure, details of gametogenesis and functioning of reproductive systems. For example, very little is known on the functioning of the spermatheca in Astigmata females (Radwan and Witaliński, 1991), as well as on events during sperm storage, migration to ovaries and details of fertilization. The same deficiency of information pertains to the role of the TCC in spermatogenesis. Recent studies on gonad development in *Histiostoma* (Witaliński et al., 2014) confirmed earlier suggestions (Witaliński et al., 1990) on TCC origin from the germinal line. However, the role of the TCC during the early stages of gametogenesis and reasons, why TCCs are absent in adult testes in some species whereas they remain in others, are still enigmatic. Further conclusions may stem from studies on species with adult testes devoid of TCC to evidence whether TCCs are present in developing gonads and, if so, when and how they disappear.

A very intriguing problem concerns oogenesis, in particular the structure of the unique intercellular bridges connecting previtellogenic oocytes with the ONC. In all studied Astigmata except Histiostomatidae, funnel shaped bridges filled tightly with some electron-dense material are present. The function of such structures as a gate controlling the in-and-out flow between the ONC and oocytes is only suggested; the same concerns the even more conspicuous and enigmatic diaphragm-crossed bridges found in *Histiostoma* species. The reasons for such profund modifications of ordinary intercellular bridges and mechanisms involved in their function should be studied in the future. Moreover, studies on ovaries in Histiostomatoidea other than *Histiostoma* could clarify whether the funnel-shaped intercellular bridges evolved from diaphragm-crossed bridges or vice versa, or both types appeared independently.

A different body of information should be collected and considered in the light of the evolution of gonads and reproduction in Astigmata. If we accept the idea that Astigmata evolved from within early Oribatida (Desmonomata: Trhypochthoniidae) (Norton et al., 1993) a number of problems must be considered. First, the gonads in contemporary Oribatida are quite different than in Astigmata (further literature: Alberti and Coons, 1999; Liana, 2004; Bergmann et al., 2008; Liana and Witaliński, 2012). For instance, oribatid ovaries are unpaired and composed of oocyte clusters connected via microtubule-rich protrusions with one or several enucleate centers (medullae). Such ovaries are evidently not of nutrimental type. Oribatid testes are usually paired, but consist of germinal and glandular parts. Second, sperm

organization in both taxa is completely different, showing peculiarities in each group (e.g. oribatid sperm contains compact, highly condensed chromatin containing mitochondrial derivatives, whereas in astigmatic spermatozoa separate chromatin threads are freely embedded in cytoplasm). Third, differences in reproductive behavior are also striking and not easy to explain: Oribatida are inseminated through stalked spermatophores deposited on the substrate, whereas Astigmata females are inseminated during copulation via an accessory inseminatory system. Moreover, thelytokous parthenogenesis is a main reproductive strategy in Desmonomata, whereas in Astigmata thelytoky seems to be secondarily evolved in some taxa only. It is necessary to emphasize, however, that in fact we know practically nothing on the reproduction of the ancestors of Astigmata since we can only study living oribatid taxa and it is at least theoretically possible that oribatid progenitors of Astigmata were much different from contemporary Desmonomata/Trhypochthoniidae.

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